19 MAY 1995

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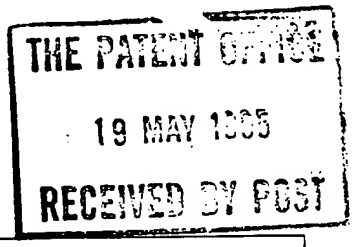

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P01/7700 25.00

08/735,385

9510141.6

Your reference
HQ/EX2190

Notes
Please type, or write in dark ink using CAPITAL letters. A prescribed fee is payable for a request for grant of a patent. For details, please contact the Patent Office (telephone 071-438 4700).

Rule 16 of the Patents Rules 1990 is the main rule governing the completion and filing of this form.

2 Do not give trading styles, for example, 'Trading as XYZ company', nationality or former names, for example, 'formerly (known as) ABC Ltd' as these are not required.

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The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

1 Title of invention

1 Please give the title of the invention

CHEMICAL COMPOUNDS

2 Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name
BIOTA SCIENTIFIC MANAGEMENT PTY LTD

Country *(and State of incorporation, if appropriate)*
AU

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please give the following details:

Address
BIOTA HOLDINGS LIMITED
P O BOX 1601
GLEN IRIS
VICTORIA 3146

UK postcode
(if applicable)

Country AU

ADP number
(if known) 6732601001

2d, 2e and 2f:
If there are further applicants please provide details on a separate sheet of paper.

❑ **Second applicant (if any)**

2d If you are applying as a corporate body please give:

Corporate name

Country *(and State of incorporation, if appropriate)*

2e If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

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Address

UK postcode
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ADP number
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3
An address for service in the United Kingdom must be supplied.
Please mark correct box

3 Address for service details

3a Have you appointed an agent to deal with your application?

Yes [X] No [ ] ➡ *go to 3b*

⬇
Please give details below

Agent's name
HELEN QUILLIN
Agent's address
GLAXO HOUSE
BERKELEY AVENUE
GREENFORD
MIDDLESEX
Postcode UB6 0NN

Agent's ADP number 647057900 1

3b:
If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name

Address

Postcode
ADP number
(if known)

Daytime telephone number *(if available)*

Additional Agents
(See Page 2 No. 3a)

NAME(S)

Alan HESKETH
Hugh B. DAWSON
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Glaxo House
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UB6 ONN
Great Britain

4 Reference number

4 Agent's or applicant's reference number *(if applicable)* HQ/EX2190

5 Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➡ *go to 6*

⬇ *please give details below*

☐ number of earlier application or patent number

☐ filing date *(day month year)*

☐ and the Section of the Patents Act 1977 under which you are claiming:

Please mark correct box

15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

6 Declaration of priority

6

If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.

Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day,month,year)*

7

The answer must be 'No' if:
- *any applicant is not an inventor*
- *there is an inventor who is not an applicant, or*
- *any applicant is a corporate bo*

7 Inventorship

7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

Please mark the correct box

Yes ☐ No ☒ ➡

A statement of Inventorship on Patents Form 7/77 will need to be filed (see Rule 15).

8

Please supply duplicates of claim(s), abstract, description and drawing(s).

8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77	1		
Claim(s)		Description	24
Abstract		Drawing(s)	

8b Which of the following documents also accompanies the application?

Please mark correct box(es)

Priority documents *(please state how many)*	
Translation(s) of Priority documents *(please state how many)*	
Patents Form 7/77 - Statement of Inventorship and Right to Grant *(please state how many)*	
Patents Form 9/77 - Preliminary Examination/Search	
Patents Form 10/77 - Request for Substantive Examination	

9

You or your appointed agent (see Rule 90 of the Patents [illegible]s 1990) must sign this request.

9 Request

I/We request the grant of a patent on the basis of this application.

Please sign here ➡

Signed Helen K Quillin Date 18/05/1995
(day month year)

H K Quillin Agent for the Applicants

A completed fee sheet should preferably accompany the fee.

Please return the completed form, attachments and duplicates where requested, together with the prescribed fee to:

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CHEMICAL COMPOUNDS

This invention relates to a new class of chemical compounds and to their use in medicine. In particular the invention concerns novel dihydropyran derivatives, methods for their preparation, pharmaceutical formulations thereof and their use as antiviral agents.

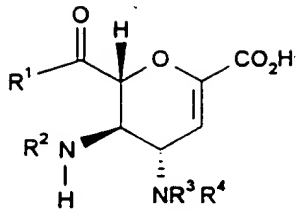
Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many microorganisms. These include bacteria such as Vibrio cholerae, Clostridium perfringens, Streptococcus pneumoniae, and Arthrobacter sialophilus, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus and Newcastle disease virus, cause diseases of enormous economic importance.

It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and its derivatives. See, e.g., Meindl et al., Virology 1974 58 457-63. International Application Publication No. WO91/16320 describes a number of analogues of DANA active both in vitro and in vivo against viral neuraminidase and useful in the treatment of influenza.

We have now found a novel class of dihydropyran derivatives which are active against the influenza virus.

The invention therefore provides, in a first aspect, compounds of formula (I)



wherein

R^1 represents OR^5, SR^5, NR^5R^6, $N(OR^5)R^6$ or $N(NR^5R^6)R^6$;

R^2 represents a group SO_2R^7 or COR^7;

R^3 represents H, C_{1-6}alkyl or $C(=NR^8)NR^9R^{10}$;

R^4 represents H or C_{1-6}alkyl;

R^5 represents H, C_{1-20}alkyl, C_{3-8}cycloalkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $CHR^{11}COR^{12}$ or C_{1-20}alkyl substituted by one or more groups selected from $NR^{13}R^{14}$, CO_2R^{13}, OR^{13}, C_{3-8}cycloalkyl and optionally substituted aryl;

Each R^6 independently represents H, C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{2-6}alkenyl, C_{2-20} alkynyl or C_{1-4}alkyl substituted by one or more groups selected from $NR^{13}R^{14}$, COR^{13}, CN, OR^{13} and optionally substituted aryl;

or R^5 and R^6 together form a C_{2-6} hydrocarbon chain which may optionally contain a group NR^{13} which chain is optionally substituted by 1, 2, 3, or 4 groups selected from oxo and C_{1-6}alkyl groups which groups may optionally be substituted by hydroxy or optionally substituted aryl;

R^7 represents C_{1-6}alkyl optionally substituted by one or more halogen atoms;

R^8, R^9 and R^{10} each independently represent H, C_{1-6}alkyl, amino, hydroxy, cyano or nitro;

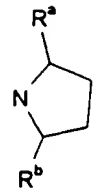
R^{11} represents the side chain of a D- or L- amino acid;

R^{12} represents $NR^{13}R^{14}$, OR^{13} or R^{13};

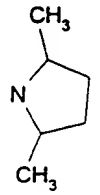
each R^{13} and each R^{14} independently represents H, C_{1-6}alkyl or optionally substituted arylC_{1-4}alkyl;

and their pharmaceutically acceptable derivatives.

Preferably R^1 represents NR^5R^6 or $N(OR^5)R^6$ where R^5 is selected from C_{1-10}alkyl such as C_{1-6}alkyl, for example methyl, ethyl, n-propyl, i-propyl or n-butyl, C_{2-6}alkenyl such as allyl, or C_{1-6}alkyl substituted by aryl such as phenylethyl, and R^6 is C_{1-6}alkyl such as methyl, ethyl, n-propyl, i-propyl or n-butyl, or R^5 and R^6 together with the nitrogen atom to which they are joined form a group



where R^a and R^b independently represent H, C_{1-6}alkyl such as methyl or hydroxymethyl, preferably H or methyl. More preferably R^5 and R^6 both represent C_{1-4}alkyl groups optionally substituted by phenyl, or NR^5R^6 represents a group



Preferably NR^3R^4 represents amino or guanidino, more preferably guanidino.

Preferably R^7 represents methyl or trifluoromethyl, more preferably methyl.

The D- or L-amino acids of which R^{11} represents the side chain may be natural amino acids, such as glycine, alanin, valine, leucine, isoleucine, serine, threonine, phenylalanine, tyrosine, tryptophan, cysteine, proline, methionine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine or histidine; or unnatural amino acids.

As used herein, alkyl includes both straight and branched chain saturated hydrocarbon groups.

As used herein, alkenyl means a straight or branched hydrocarbon chain containing one or more carbon-carbon double bonds.

Aryl means aromatic carbocyclic (e.g. phenyl) and heterocyclic (e.g. pyridyl, imidazolyl) groups. Particularly suitable is phenyl. When aryl groups are optionally substituted, suitable substituents include C_{1-4}alkyl, C_{1-4}alkoxy, halo, hydroxy, cyano, nitro, trifluoromethyl, amino, phenyl and benzyl.

By "a pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, ester, or salt of such ester, of a compound of formula (I) or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) a compound of formula (I) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compounds of formula (I). Of particular interest as such derivatives are compounds modified at the carboxyl function, hydroxyl functions or at amino groups. Thus compounds of interest include alkyl (such as methyl, ethyl or propyl e.g. isopropyl) or aryl (e.g. phenyl, benzoyl) esters and acetyl esters of the compounds of formula (I).

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compounds of formula (I) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene- p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable may be useful in the preparation of salts useful as intermediates in obtaining compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and NR_4^+ (where R is C_{1-4}alkyl) salts.

Preferred compounds include :-

(4S, 5R,6R)-5-acetylamino-6-dimethylcarbamoyl-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-guanidino-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-amino-6-(2,5-dimethyl-pyrrolidine-1-carbonyl)-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-amino-6-dipropylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-6-dipropylcarbamoyl-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-6-dibutylcarbamoyl-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-amino-6-(phenylethyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-6-(phenylethyl-propyl-carbamoyl)-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-amino-6-(butylpropylcarbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-amino-6-diethylcarbamoyl 5,6-dihydro-4H-pyran-2-carboxylic acid;

(4S, 5R,6R)-5-acetylamino-4-amino-6-(ethylpropylcarbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid;

and pharmaceutically acceptable derivatives thereof.

References hereinafter to a compound of the invention includes the compounds of formula (I) and pharmaceutically acceptable derivatives thereof.

The compounds of formula (I) possess antiviral activity. In particular these compounds are inhibitors of viral neuraminidase of orthomyxoviruses and paramyxoviruses in particular influenza neuraminidase, for example the viral neuraminidase of influenza A and B.

Compounds of the invention have been tested for their ability to inhibit the multiplication of influenza virus in a plaque reduction assay essentially as described in WO91/16320. Typically, IC_{50} values for influenza A and B were less than 50μg/ml.

There is thus provided in a further aspect of the invention a compound of formula (I) or a pharmaceutically acceptable derivative thereof for use as an active therapeutic agent in particular as an antiviral agent for example in the treatment of influenza virus infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, for example an influenza virus infection in a mammal including man comprising administration of an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

There is also provided in a further or alternative aspect use of a compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

The compounds of the invention may also be used in disagnostic methods, in particular methods for the detection of influenza virus. For use in such methods it may be advantageous to link a compound of the invention to a detachable label.

The amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected but also with the route of

administration, the nature of the condition being treated and the age and condition of the patient and will ultimately be at the discretion of the attendant physician or veterinarian. In general however a suitable dose will be in the range of from about 0.1 to 750mg/kg of bodyweight per day, preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compounds are also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given 1-4 times daily and continued for 3-7, e.g. 5 days post infection depending upon the particular compound used.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage form for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, a compound of the invention may be administered as the raw chemical it is preferable to present the active ingredient as a pharmaceutical formulation

The invention thus further provides a pharmaceutical formulation comprising a compound of formula (1) or a pharmaceutically acceptable salt or derivative thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form

suitable for administration to the respiratory tract (including the nasal passages) for example by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compounds according to the invention may also be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For topical administration to the epidermis the compounds according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated

with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes; foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Preferably the compounds of the invention will be administered to the respiratory tract.

For administration to the respiratory tract (including intranasal administration) the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for administration to the respiratory tract, including inhalation via the nose and/or mouth using a nebuliser or an inhaler.

Thus in general the compounds may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous for example prepared from water alone (for example sterile or pyrogen-free water) or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol, polyethlene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (e.g. Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluroroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g. gelatin or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the compound will generally have a small particle size for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired, formulations adapted to give sustained release of the active ingredient may be employed.

The compounds of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compounds of the invention may be employed with other antiviral agents. The invention thus provides in a further aspect a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof together with another therapeutically active agent, in particular an antiviral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

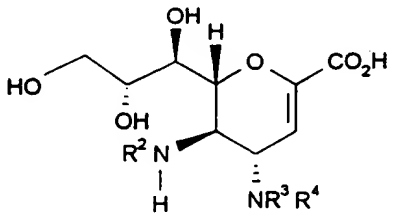
Suitable therapeutic agents for use in such combinations include other anti-infective agents, in particular anti-bacterial and anti-viral agents such as those used to treat respiratory infections. For example, other compounds effective against influenza viruses, such as amantadine, rimantadine and ribavirin, may be included in such combinations.

The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compounds of the invention are used with a second therapeutic agent active against the same virus the dose of each compound may either be the same as or differ from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compounds of formula (I) and their pharmaceutically acceptable salts and derivatives may be prepared by the methods described below in which R, R^1, R^2, R^3 R^4, R^5 and R are as defined for formula (I) unless otherwise specified. The methods outlined below form a further aspect of the invention.

In one such process (A) compounds of formula (I) wherein R^1 is hydroxy may be prepared from compounds of formula (II)



(II)

or a suitably protected derivative thereof, by oxidative cleavage of the glycerol sidechain, followed, if necessary, by deprotection.

Conveniently the oxidative cleavage is carried out in two steps. Suitably the first step is effected using a periodate, such as, for example, sodium periodate, conveniently in a suitable solvent, such as an aqueous organic solvent, for example, aqueous methanol. Suitable reagents for the second step of the oxidative cleavage include chlorites, for example sodium chlorite, suitably in the presence of a buffering agent, such as an alkali or alkline earth metal phosphate for example, potassium phosphate , in an aqueous organic solvent, such as an aqueous mixture of an alcohol and a hydrocarbon, for example, an aqueous mixture of t-butanol and cyclohexene.

Compounds of formula (I) wherein R^1 represents NR^5R^6 may be prepared from the corresponding compounds of formula (I) wherein R^1 represents hydroxy by reaction with an amine of formula HNR^5R^6. Suitably the hydroxy group is activated prior to reaction with the amine. Suitable methods of activation will be readily apparent to those skilled in the art and include, for example, conversion to a pentafluorophenoxy group. The amination is conveniently effected in a suitable organic solvent such as an ether, for example, tetrahydrofuran.

Other compounds of formula (I) may be prepared by interconversion of different compounds of formula (I). For example, compounds wherein R^3 and

R^4 are other than H may be prepared by derivatisation of the corresponding compound wherein R^3 and/or R^4 are H.

As will be appreciated by those skilled in the art it may be necessary or desirable at any stage in the above described processes to protect one or more sensitive groups in the molecule to prevent undesirable side reactions; the protecting group may be removed at any convenient subsequent stage in the reaction sequence.

The protecting groups used in the preparation of compounds of formula (I) may be used in conventional manner. See for example 'Protective Groups in Organic Chemistry' Ed. J. F. W. McOmie (Plenum Press 1973) or 'Protective Groups in Organic Synthesis' by Theodora W Greene (John Wiley and Sons 1981).

Conventional amino protecting groups may include for example aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups; and acyl groups such as N-benzyloxycarbonyl or t-butoxycarbonyl.

Hydroxy groups may be protected, for example, by aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups, acyl groups, such as acetyl, silicon protecting groups, such as trimethylsilyl groups, or as tetrahydropyran derivatives.

Removal of any protecting groups present may be achieved by conventional procedures.

Where it is desired to isolate a compound of the invention as a salt, for example as an acid addition salt, this may be achieved by treating the free base of general formula (I) with an appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

The present invention is further described by the following examples which are for illustrative purposes only and should not be construed as a limitation of the invention.

Example 1
(4S,5R,6R)-5-Acetylamino-4-amino-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt.

a) (4S,5R,6R)-5-Acetylamino-4-(tert-butoxycarbonylamino)-6-(1S,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester

To a suspension of (4S,5R,6R)-5-acetylamino-4-amino-6-(1S,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trihydrate (8.25g,) in dioxan/water (2:1v/v, 75ml) was added sodium bicarbonate (2.6g) and di-*t*-butyl pyrocarbonate (6.76g) and the reaction was stirred at 23°C for 18 hours. The resulting solution was acidified to pH6 using 2N hydrochloric acid and to this was added a solution of diphenyldiazomethane in dichloromethane (125ml of a 0.29M solution). This was stirred rapidly for 24 hours whilst maintaining the pH at approximately 6 using 2N hydrochloric acid. The resulting suspension was filtered and the solid was dried *in vacuo* to give the title compound. (10.9g); ^{1}H NMR (250 MHz, D_6-DMSO) 8.13 (1H, d, J=8Hz), 7.49-7.25 (10H, m), 7.15 (1H, d, J=8.75Hz), 6.87 (1H, s), 5.88 (1H, m), 4.63 (2H, m), 4.47 (1H, m), 4.34 (1H, m), 4.18 (1H, m), 3.91 (1H, m), 3.67 (2H, m), 3.40 (2H, m), 1.88 (3H, s), 1.41 (9H, s); Mass spec (Low resolution): MH^+=557, MH^+-BOC=457; Mass analysis:
C_{29} H_{36} N_2 O_9. 1.5H_2 O. Required: C,59.68; H, 6.74; N, 4.80. Found: C, 59.65; H, 6.61; N, 4.84.

b). (2R,3R,4S)-3-Acetylamino-4-(tert-butoxycarbonylamino)-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester

(4S,5R,6R)-5-Acetylamino-4-(tert-butoxycarbonylamino)-6-(1S,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester (8.0g) was dissolved in methanol/water (5:1 v/v, 180ml). To this was added sodium periodate (6.93g) and the reaction was stirred at 23°C for 3 hours. The solid was removed by filtration and the filtrate was evaporated *in vacuo* to give a white solid.

The solid obtained by evaporation of the filtrate was suspended in *t*-butanol (70ml) and cyclohexene (10ml) and stirred rapidly at 23°C. To this was added a solution of sodium chlorite (10.7g) and potassium dihydrogen

orthophosphate (10.7g) in water (50ml). After 18 hours a pale yellow solution was obtained which was acidified using 2N hydrochloric acid. This was extracted into ethyl acetate (3 x 200ml) and dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* and the residue was triturated with diethyl ether. The solid was collected by filtration and dried to give the title compound. (5.1g). ^{1}H NMR (250 MHz, D_6-DMSO).13.1 (1H, broad s), 7.96 (1H, d, J=8.75Hz), 7.48-7.26 (10H, m), 6.92 (1H, s), 6.83 (1H, d, J=6.25Hz), 6.06 (1H, d, J=3Hz), 4.57 (1H, d, J=6.25Hz), 4.28 (1H, m), 4.15 (1H, m), 1.79 (3H, s), 1.38 (9H, s): Mass analysis: $C_{27}H_{30}N_2O_8$. $0.25H_2O$.Requires: C, 62.96; H, 5.97; N, 5.44.Found: C, 62.85; H, 5.93; N, 5.52; Mass Spec (Low resolution): MH^+= 511

c) (2R,3R,4S)-3-Acetylamino-4-(tert-butoxycarbonylamino)-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester 2(2,3,4,5,6-pentafluoro-phenyl) ester

(2R,3R,4S)-3-Acetylamino-4-(tert-butoxycarbonylamino)-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester (3.36g) was dissolved in dry dimethylformamide (10ml) and pyridine (0.632g) under nitrogen and stirred at 23°C. To this was added pentafluorophenyl trifluoroacetate (2.02g), after 3 hours the reaction mixture was diluted with ethyl acetate (250ml) and washed with dilute hydrochloric acid (3 x 50ml), dilute sodium bicarbonate solution (3 x 50ml) and brine (50ml). The organic phase was dried over anhydrous magnesium sulphate and the solvent was removed *in vacuo* to give the title compound as an off-white foam. (4.353g); ^{1}H NMR (250MHz, DMSO)

8.22 (1H, d, J=8Hz), 7.50-7.27 (10H, m), 7.06 (1H, m), 6.94 (1H, s), 6.18 (1H, d, J=3.8Hz), 5.25 (1H, d, J=6.8Hz), 4.42 (1H, m), 4.29 (1H, m), 1.82 (3H, s), 1.38 (9H, s).

d). (4S,5R,6R)-5-Acetylamino-4-(tert-butoxycarbonylamino)-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester

To (2R,3R,4S)-3-acetylamino-4-(tert-butoxycarbonylamino)-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester 2-(2,3,4,5,6-pentafluoro-phenyl) ester (1.18g) in dry tetrahydrofuran (13ml) was added N-methyl-propylamine (0.152g) and the reaction was stirred at 23°C for 4 hours. The solvent was

removed *in vacuo* and the residue was chromatographed over silica (Merck 9385, 150g) using medium pressure (~ 4 psi) and ethyl acetate as the eluant. The required fractions were combined and the solvent removed *in vacuo* to give the title compound as a white foam. (0.85g); ^{1}H NMR (250MHz, D6-DMSO) 8.10-7.98 (1H, m), 7.48-7.26 (10H, m), 6.91 (1H, s), 6.58 (1H, m), 6.03 (1H, m), 5.13 (1H, m), 4.34 (1H, m), 4.03 (1H, m), 3.37 (2H, m), 3.07+2.81 (3H, s), 1.77 (3H, s), 1.64-1.22 (2H, m), 1.37 (9H, s), 0.81 (3H, m): Mass spec (Low resolution): MH^+=566, MH^+-BOC=466; Mass analysis: $C_{31}H_{39}N_3O_7$. $0.5C_6HF_5O$. Required: C, 62.09; H, 6.05; N, 6.39. Found: C, 61.85; H, 6.07; N, 6.43.

e)(4S,5R,6R)-5-Acetylamino-4-amino-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

(4S,5R,6R)-5-Acetylamino-4-(tert-butoxycarbonylamino)-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester (0.10g) was dissolved in dichloromethane (1ml) and trifluoroacetic acid (1ml) and left to stand at 23°C for 3 hours. The solvent was removed *in vacuo* and the residue was triturated using diethyl ether (30ml). The resulting solid was collected by filtration and dried to give the title compound as a white solid. (0.064g): ^{1}H NMR (250MHz, D_2O) 5.99 (1H, m), 5.24 (1H, m), 4.52 (1H, m), 4.25 (1H, m), 3.59-3.18 (2H, m), 3.18+2.97 (3H, s), 2.02 (3H, s), 1.72-1.46 (2H, m), 0.87 (3H, m); Mass Analysis: $C_{15}H_{22}F_3N_3O_7$. $0.25H_2O$. Required C, 43.12; H, 5.43; N, 10.06; Found C, 42.99; H, 5.60; N, 10.17; Mass Spec. (Low Resolution) MH^+ = 300.

Example 2

(4S, 5R, 6R)-5-Acetylamino-4-guanidino-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carbxylic acid trifluoroacetate salt.

a) (4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester

(4S,5R,6R)-5-Acetylamino-4-(tert-butoxycarbonylamino)-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester (0.83g) was dissolved in a solution of hydrogen chloride in dioxane (10ml of a 4.0N solution) and stirred under nitrogen for 30 minutes. The solvent was removed

in vacuo to give an off-white foam. This was suspended in tetrahydrofuran (10ml) and dimethylformamide (5ml). To this was added triethylamine (0.42ml) and (tert-butoxycarbonylimino)-pyrazol-1-yl-methyl-carbamic acid tert-butyl ester (0.682g) and the reaction was stirred at 23°C for 18 hours. The reaction was partitioned between ethyl acetate and 1N hydrochloric acid. The organic phase was dried over anhydrous magnesium sulphate and the solvent was removed *in vacuo*. The residue was chromatographed over silica (Merck 9385, 30g) using medium pressure (~4psi) and cyclohexane/ethyl acetate (1:1 v/v) as eluant. The required fractions were combined and the solvent was removed *in vacuo* to give the title compound as a white foam. (0.607g); ^{1}H NMR (250MHz, DMSO) 11.41 (1H, s), 8.63 (1H, m), 7.40-7.29 (10H, m), 6.99 (1H, s), 6.12 (1H, m), 5.85 (1H, m), 5.16 (1H, m), 5.04 (1H, m), 4.33 (1H, m), 3.45 (2H, m), 3.17+2.89 (3H, s), 1.98 (3H, s), 1.69-1.42 (2H, m), 1.49 (18H, s), 0.87 (3H, m); Mass Spec (Low resolution):MH^+ = 708, MH^+ - BOC= 608, MH^+-2BOC = 508;
Mass analysis: $C_{37}H_{49}N_5O_9$. $0.75H_2O$. Required: C, 61.61; H, 7.06; N, 9.71. Found: C, 61.81; H, 6.85; N, 9.73.

b). (4S,5R,6R)-5-Acetylamino-4-guanidino-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

(4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-(methyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester (0.11g) was dissolved in dichloromethane (1ml) and trifluoroacetic acid (1ml) and left to stand for 3 hours. The solvent was removed *in vacuo* and the residue was triturated using diethyl ether. The solid was collected by filtration and dried to give the title compound as a white solid. (0.061g) ^{1}H NMR (250MHz, D_2O)6.00 (1H, d, J=4Hz), 5.34 (1H, d, J=6.3Hz), 4.43-4.29 (2H, m), 3.56-3.13 (2H, m), 3.13,2.93 (3H, 2xs), 2.00 (3H, s), 1.79-1.44 (2H, m), 0.88 (3H, m); Mass analysis:$C_{16}H_{24}F_3N_5O_7$. Required C, 42.20; H, 5.31; N15.38. Found C, 42.24; H, 5.45; N, 15.09; Mass Spec (Low resolution):MH^+ = 342.

The following Examples 3-14 were similarly prepared by the methods described in Examples 1 and 2:

Example 3

(4S,5R,6R)-5-acetylamino-4-amino-6-propylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}H NMR (250MHz, D_2O) 6.00 (1H, d, J=2.5Hz), 4.54 (1H, d, J=10Hz), 4.42 (1H, t, J=9.4Hz), 4.27 (1H, dd, J=2.5, 9.4Hz), 3.18 (2H, t, J=7Hz), 2.03 (3H, s), 1.51 (2H, sextet, J=7Hz), 0.88 (3H, t, J=7Hz).

Example 4

(4S,5R,6R)-5-Acetylamino-4-guanidino-6-propylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}H NMR (250MHz, D_2O) 5.88 (1H, d, J=4.5Hz), 4.83 (1H, d), 4.51 (1H, t, J=4.4Hz), 4.28 (1H, t, J=4.4Hz), 3.17 (2H, m), 2.02 (3H, s), 1.49 (2H, m, J=7Hz), 0.88 (3H, t, J=7Hz).

Mass spec (Low resolution): MH^+=328.

Mass analysis

$C_{15}H_{22}F_3N_5O_7$. $0.15C_4H_{10}O$. Required: C,41.16; H, 5.27; N, 15.39. Found: C, 41.24; H, 5.41; N, 15.12.

Example 5

(4S,5R,6R)-5-acetylamino-4-amino-6-dipropylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}H NMR (250MHz, DMSO) 8.20 (1H, d), 6.01 (1H, d), 5.14 (1H, d), 4.37 (1H, q), 4.22 (1H, m), 3.55 (2H, m), 3.32 (1H, m), 3.15 (1H, m), 1.80 (3H, s), 1.65-1.40 (4H, m), 0.85 (6H, m). Mass spec (Low resolution):MH^+=328 Mass analysis: $C_{17}H_{25}F_3N_3O_7$. H_2O. Required: C, 44.44; H, 6.14; N, 9.15. Found: C, 44.72; H, 6.22; N, 9.35.

Example 6

(4S,5R,6R)-5-acetylamino-4-amino-6-dibutylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}H NMR (250MHz, D_2O) 5.95 (1H, broad d), 5.16 (1H, d, J=10Hz), 4.48 (1H, t, J=8.5Hz), 4.27 (1H, dd, J=1, 8.5Hz), 3.70-3.10 (4H, m), 2.01 (3H, s), 1.70-1.20 (8H, m), 0.90 (6H, t, J=7.5Hz).Mass spec (Low resolution): MH^+=356

Mass Analysis: $C_{19}H_{30}F_3N_3O_7$. Required: C, 49.30; H, 6.58; N, 9.17. Found: C, 48.61; H, 6.44; N, 8.95.

Example 7

(4S,5R,6R)-5-acetylamino-4-amino-6-(decyl-methyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}H NMR (250MHz, DMSO, rotamers)
8.1 (1H, two d), 5.8 (1H, d), 5.0 (1H, two d), 4.1-3.3 (4H, m), 3.0+2.8 (3H, two s), 1.8 (3H, s), 1.6-1.1 (16H,m), 0.9 (3H, t).Mass spec (Low resolution): MH^+=398
Mass analysis
$C_{22}H_{36}F_3N_3O_7$. Required: C, 51.7; H, 7.0; N, 8.2. Found: C, 52.36; H, 7.27; N, 8.68.

Example 8
(4S,5R,6R)-5-Acetylamino-4-amino-6-(phenethyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt
^{1}H NMR (250MHz, D_2O) 7.4-7.2 (5H, m), 5.96+5.80 (1H, 2xd), 5.17 (1H, d, J=7.5Hz), 4.6-2.8 (15H, m), 2.01+1.99 (3H, 2xs), 1.7-1.5 (2H, m), 0.86 (3H, t, J=7.5Hz).
Mass spec (Low resolution): MH^+=390
Mass analysis
$C_{22}H_{28}F_3N_3O_7$. Requires: C, 52.68; H, 5.61; N, 8.35. Found: C, 53.05; H, 5.74; N, 8.41.

Example 9
(4S,5R,6R)-5-acetylamino-4-amino-6-(methoxy-methyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt
^{1}H NMR (250MHz, D_2O, rotamers) 6.00 (1H, d, J=2.5Hz), 5.23+5.07 (1H, d, J=10Hz), 4.48 (1H, t, J=10Hz), 4.28 (1H, dd, J=2.5, 10Hz), 3.80+3.71 (3H, two s), 3.48+3.26 (3H, two s), 2.04+2.02 (3H, two s).
Mass spec (Low resolution):MH^+=288
Mass analysis $C_{13}H_{18}F_3N_3O_8$. $0.75H_2O$. Required: C, 37.64; H, 4.74; N, 10.13. Found: C, 37.65; H, 4.75; N, 9.95.

Example 10
(4S,5R,6R)-5-acetylamino-4-amino-6-(2,5-dimethyl-pyrrolidine-carbonyl)-5,6-dihydro-4H-pyran-2-carboxylic acid tifluoroacetate salt

^{1}H NMR (250MHz, D_2O, rotamers) 5.95 (1H, d), 5.10+4.98 (1H, d), 4.58+4.48 (1H, t), 4.4-4.2 (2H, m), 4.0 (1H, m), 2.2-1.9 (3H, s + 2H, m), 1.9-1.7 (2H, m), 1.35-1.22 (6H, m).
Mass spec (Low resolution): MH^+=326
Mass analysis
$C_{17}H_{24}F_3N_3O_7$. Required: C,47.47; H, 5.51; N, 9.56. Found: C, 46.91; H, 5.77; N, 9.61.

Example 11
(4S,5R,6R)-5-Acetylamino-4-guanidino-6-dimethylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}H NMR (250MHz, D_2O)6.01 (1H, d, J=3Hz), 5.34 (1H, d, J=6Hz), 4.38 (2H, m), 3.15 (3H, s), 2.95 (3H, s), 2.01 (3H, s).
Mass spec (Low resolution):MH^+=314
MMass analysi$C_{14}H_{20}F_3N_5O_7.0.1H_2O$. Requires: C, 39.62; H, 4.89; N, 16.04. Found: C, 39.57; H, 4.85; N, 15.81.

Example 12
(4S,5R,6R)-5-Acetylamino-4-amino-6-(butylpropylcarbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt
^{1}Hnmr (DMSO,250MHz) 0.85(6H,m), 1.25(2H,m), 1.35-1.70(4H,m), 1.81(3H,s), 2.95-3.60(4H,m),4.04(1H,d of d), 4.18(1H,q), 4.98(1H,d of d), 5.81(1H,s), 8.18(1H,d,J=7.5Hz)
Mass spec (Low resolution)MH^+= 342
Mass analysis $C_{18}H_{28}F_3N_3O_7$.Requires: C, 47.47; H, 6.20; N, 9.23. Found: C, 47.63; H, 6.23; N, 9.15.

Example 13
(4S,5R,6R)-Acetylamino-4-amino-6-diethylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}Hnmr (DMSO,250MHz) 1.00(3H,t,J=7.5Hz), 1.15(3H,t,J=7.5Hz), 1.80(3H,s), 3.10-3.60(4H,m), 4.07(1H,d of d), 4.23(1H,q,J=7.5Hz), 5.00(1H,d,J=7.5Hz), 5.88(1H,d,J=2.5Hz), 8.18(1H,d,J=7.5Hz)
Mass spec (Low resolution):MH^+= 300

Mass analysis$C_{13}H_{21}N_3O_5$. 1.2 $C_2HF_3O_2$. Requires: C, 42.41; H, 5.13; N, 9.63. Found: C, 42.33; H, 5.25; N, 9.66.

Example 14

(4S,5R,6R)-5-Acetylamino-4-amino-6-(ethylpropylcarbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^{1}Hnmr (DMSO,250MHz)0.83(3H,m), 1.00(1H,t,J=7.5Hz), 1.15(2H,t,J=7.5Hz), 1.40-1.63(2H,m), 1.80(3H,s), 3.00-3.60(4H,m), 4.08(1H,m), 4.23(1H,m,J=7.5Hz), 5.00(1H,t,J=7.5Hz), 5.87(1H,t), 8.18(1H,m)

Mass analysis$C_{16}H_{24}F_3N_3O_7$. H_2O. Requires: C, 43.15; H, 5.88; N, 9.43. Found: C, 43.41; H, 5.73; N, 9.22.

Example 15

(4S, 5R, 6R)-5-Acetylamino-4-guanidino-6-dipropylcarbamoyl- 5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

To a suspension of (4S,5R,6R)-5-acetylamino-4-amino-6-(1S,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trihydrate (7.8g) in methanol (60ml) was added (tert-butoxycarbonylimino)-pyrazol-1-yl-methyl-carbamic acid tert-butyl ester (7.05g,) and triethylamine (4.18ml). The reaction was stirred at 23°C for 18 hours. Diethyl ether (200ml) was added and the resultant solid was collected by filtration to give a white solid. This was suspended in a solution of diphenyldiazomethane in dichloromethane (48ml of a 0.47M solution), acidified using 2N hydrochloric acid and stirred rapidly for 18 hours. The organic phase was separated and the solvent removed *in vacuo* to give a purple foam. This was chromatographed over silica gel (Merck 9385, 80g) using medium pressure (~4psi) and ethyl acetate as eluant. The required fractions were combined and the solvent removed *in vacuo* to give the title compound as a white foam. (11.1g) ^{1}H NMR (250MHz, DMSO) 11.40 (1H, s), 8.28 (1H, d, J=7.5Hz), 8.21 (1H, d, J=8Hz), 7.49-7.27 (10H, m), 6.88 (1H, s), 5.97 (1H, d, J=2.5Hz), 4.88 (1H, m), 4.68 (1H, d, J=6Hz), 4.63 (1H, d, J=6Hz), 4.36 (1H, t, J=6Hz), 4.25 (1H, m), 4.12 (1H, m), 3.69 (2H, m), 3.44 (2H, m), 1.87 (3H, s), 1.48 (9H, s), 1.42 (9H, s); Mass analysis: $C_{35}H_{46}N_4O_{11}$. $0.5C_4H_8O_2$. $0.3H_2O$. Required: C, 59.39; H, 6.82; N, 7.49. Found: C, 59.41; H, 6.46; N, 7.43.

b)(2R,3R,4S)-3-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester

To a solution of (4S,5R,6R)-5-acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-quanidino]-6-(1S,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester (9.19g) in methanol/water (5:1 v/v, 120ml) was added sodium periodate (6.38g) and the reaction was stirred at 23°C for 1.5 hours. The solid was removed by filtration and the filtrate was evaporated *in vacuo* to give a white solid. This was suspended in t-butanol (55ml) and cyclohexene (7.7ml). To this was added a solution of sodium chlorite (8.14g) and potassium dihydrogen orthophosphate (8.14g) in water (44ml) and the reaction was stirred at 23°C for 2 hours. The reaction was acidified and extracted using ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulphate and the solvent was removed *in vacuo* to give a tan foam. This was dissolved in diethyl ether and petroleum ether (40-60) was added the solid was collected by filtration to give the title compound as a white solid. (6.00g) ^{1}H NMR (250MHz, DMSO) 11.39 (1H, s), 8.27-8.17 (2H, m), 7.50-7.27 (10H, m), 6.96 (1H, s), 6.19 (1H, d, J=5Hz), 4.82 (1H, d, J=4Hz), 4.53 (1H, m), 4.43 (1H, m), 1.83 (3H, s), 1.47 (9H, s), 1.41 (9H, s); Mass analysis: $C_{33}H_{40}N_4O_{10}$. $0.75C_4H_8O_2$. Required: C, 60.16; H, 6.45; N, 7.79. Found: C, 58.95; H, 6.21; N, 7.80 Mass spec)Low resolution) : MH^+ =653.;

c)(2R,3R,4S)-3-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester 2-(2,3,4,5,6-pentafluoro-phenyl) ester

To a solution of (2R,3R,4S)-3-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester (4.4g) in dry dimethylformamide (10ml) and pyridine (0.70ml) was added pentafluorophenyl trifluoroacetate (1.27ml) and the reaction was stirred at 23°C for 1 hour. More pyridine (0.70ml) and pentafluorophenyl trifluoroacetate (1.27ml) was added and the reaction was stirred for a further 2 hours. The reaction mixture was diluted using ethyl acetate and washed consecutively using 1N hydrochloric acid, saturated sodium bicarbonate solution and brine. The organic phase was dried over anhydrous magnesium

sulphate and the solvent was removed *in vacuo* to give the crude title compound as a tan foam, (8.11g); ^{1}H NMR (250MHz, DMSO)
9.97 (1H, broad s), 7.49-7.27 (11H, m), 6.91 (1H, s), 6.52 (1H, m), 4.93 (1H, m), 4.58 (1H, m), 4.47 (1H, m), 1.88 (3H, s), 1.42 (18H, s).

d).(4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-dipropylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester

To a solution of (2R,3R,4S)-3-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-3,4-dihydro-2H-pyran-2,6-dicarboxylic acid 6-benzhydryl ester 2-(2,3,4,5,6-pentafluoro-phenyl) ester (0.70g) in dry tetrahydrofuran (5ml) was added dipropylamine (0.141g) and the reaction was stirred at 23°C for 4 hours. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (Merck 9385, 50g) using medium pressure (~4psi) and cyclohexane/ethyl acetate (1:1 v/v) as the eluant. The required fractions were combined and the solvent removed *in vacuo* to give the title compound as a pale yellow foam. (0.341g). ^{1}H NMR (250MHz, DMSO) 11.46 (1H, s), 8.27 (1H, d, J=6.25Hz), 8.16 (1H, d, J=7.5Hz), 7.48-7.27 (10H, m), 6.97 (1H, s), 6.14 (1H, d, J=5Hz), 5.18 (1H, m), 4.67 (1H, m), 4.10 (1H, m), 3.61-3.24 (3H, m), 3.14 (1H, m), 1.84 (3H, s), 1.60-1.34 (4H, m), 1.46 (9H, s), 1.40 (9H, s), 0.85 (3H, t, J=7Hz), 0.74 (3H, t, J=7Hz); Mass Analysis;
$C_{39}H_{53}N_5O_9$. $1.0C_6HF_5O$. Required: C, 58.75; H, 5.92; N, 7.61. Found: C, 58.64; H, 5.75; N, 7.61.

e). (4S,5R,6R)-5-Acetylamino-4-guanidino-6-dipropylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

(4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-dipropylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid benzhydryl ester (0.32g) was dissolved in dichloromethane (2ml) and trifluoroacetic acid (2ml) and left to stand at 23°C for 2 hours. The solvent was removed *in vacuo* and the residue triturated with diethyl ether (20ml). The resulting solid was collected by filtration and dried to give the title compound as an off-white solid. (0.158g)

^{1}H NMR (250MHz, D_2O) 5.97 (1H, d, J=3.8Hz), 5.29 (1H, d, J=6.3Hz), 4.41 (1H, dd, J=3.8, 6.3Hz), 4.30 (1H, t, J=6.3Hz), 3.63-3.08 (4H, m), 1.99 (3H, s), 1.66+1.51 (4H, m) Mass Spec (Low resolution):MH^+=370:Mass

analysis:$C_{18}H_{28}F_3N_5O_7$. Required: C, 44.72; H,5.84; N,14.49. Found: C, 44.72; H, 5.87; N, 13.99.

The following Examples 16-18 were similarly prepared using the method described in Example 15:

Example 16
(4S,5R,6R)-5-Acetylamino-4-guanidino-6-dibutylcarbamoyl-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^1H NMR (250MHz, D_2O) 5.99 (1H, d, J=3.8Hz), 5.29 (1H, d, J=6.3Hz), 4.39 (1H, m), 4.28 (1H, m), 3.58-3.12 (4H, m), 2.00 (3H, s), 1.75-1.13 (8H, m), 0.89 (6H, m); Mass spec (Low resolution): MH^+=398 Mass analysis:$C_{20}H_{32}F_3N_5O_7$. Requires: C, 46.96; H, 6.31; N, 13.69. Found: C, 46.26; H, 5.94; N, 13.26.

Example 17

(4S,5R,6R)-5-Acetylamino-4-guanidino-6-(phenethyl-propyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt
^1H NMR (250MHz, D_2O) 7.5-7.2 (5H, m), 5.98+5.85 (1H, 2xd), 5.28 (1H, d, J=6.5Hz), 4.6-2.8 (15H, m), 2.00+1.97 (3H, 2xs), 1.6-1.45 (2H, m), 0.86 (3H, t, J=7.5Hz).Mass spec (Low resolution) MH^+=432; Mass analysis $C_{23}H_{30}F_3N_5O_7$. Requires: C, 50.64; H, 5.54; N, 12.84. Found: C, 50.01; H, 5.50; N, 12.10.

Example 18
(4S,5R,6R)-5-Acetylamino-4-guanidino-6-(decyl-methyl-carbamoyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate salt

^1H NMR (250MHz, DMSO) 8.68+8.59 (1H, 2xd), 7.23 (1H, m), 5.81 (1H, d, J=2.5Hz), 5.19 (1H, m), 4.20-4.00 (2H, m), 3.40-3.12 (2H, m), 3.03+2.81 (3H, 2xs), 1.87 (3H, 2xs), 1.42 (2H, m), 1.24 (16H, m), 0.87 (3H, m);Mass analysis $C_{23}H_{38}F_3N_5O_7$. $0.33H_2O$. Requires: C, 49.37; H, 6.96; N, 15.52. Found: C, 49.41; H, 6.88; N, 12.51.

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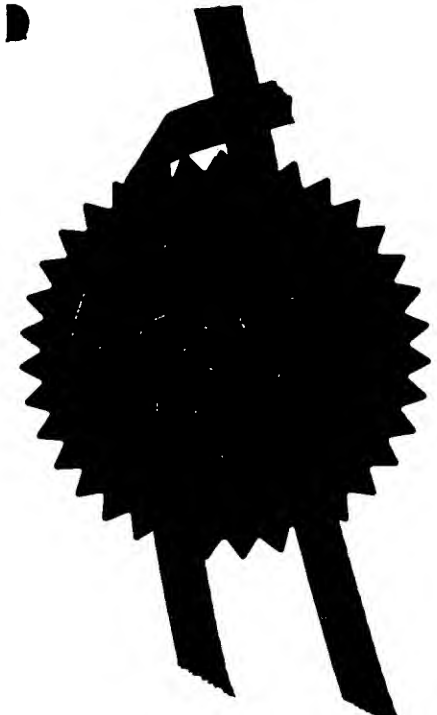

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